TRIMORPHIC INCOMPATIBILITY IN MEXICAN POPULATIONS OF *PONTEDERIA SAGITTATA* PRESL. (PONTEDERIACEAE)

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SUMMARY

Populations of *Pontederia sagittata* from the northern coastal plain of Mexico possess a tristylous breeding system. Styles and stamens are reciprocally arranged in the three floral morphs. Differences in anther size, pollen size and pollen production are associated with the three anther levels. Controlled pollinations performed in the glasshouse indicate that physiological self-incompatibility accompanies floral trimorphism. Pollinations between morphs with anthers and stigmas at equivalent heights are highly productive of seed in comparison with self- or intra-morph pollinations. In each floral morph, substantial differences in seed production result from self-pollination with alternate anther levels. Overall, the mid-styled morph exhibits weak self-incompatibility whereas the long- and short-styled morphs are more strongly self-incompatible. In all pollen–stigma combinations pollen germinated readily and pollen tubes penetrated stigmatic tissue. Inhibition of pollen tubes occurs in the style and possibly the ovary. The rate of pollen tube growth differs between anther levels following self-pollination.

The three floral morphs were represented in eight populations examined although there was a tendency for the long-styled morph to be under-represented. Strong pollen trimorphism allows the origin of pollen deposited on naturally pollinated stigmas to be determined. Considerable variation in the amount and composition of pollen deposited on stigmas by syrphid flies was recorded. The likelihood of legitimate pollination appears to be highest in the long-styled morph, intermediate in the mid-styled morph and lowest in the short-styled morph. Tristyly in *P. sagittata* is compared with the closely related *P. cordata* and *P. rotundifolia*. Implications of weak self-incompatibility in the mid-styled morph for the breakdown of tristylous breeding systems in the Pontederiaceae are discussed.

INTRODUCTION

Perhaps the most complex outbreeding systems in the flowering plants are those which involve heteromorphic self-incompatibility. Populations of heterostylous plants usually contain two (distyly) or three (tristyly) mating types which differ principally in the lengths of styles and stamens, pollen size, and incompatibility relationships. While distyly is widely distributed among angiosperm families, tristyly only occurs in the Lythraceae, Oxalidaceae and Pontederiaceae (Vuilleumier, 1967). Examination of the morphological features and genetical basis of heterostyly has received considerable attention during the past century (reviewed in Ganders, 1979), but until recently there has been little detailed work on the population biology of heterostylous plants.

In the Pontederiaceae, tristyly occurs in *Pontederia* (four species) and *Eichhornia* (three species). Comparative studies indicate that the expression of tristyly in the two genera is strikingly different. In the two *Pontederia* species which have been examined experimentally (*P. rotundifolia* L., *P. cordata* L.), tristyly is accompanied by self-incompatibility, strong pollen trimorphism and populations

which usually contain the three floral morphs (Ornduff, 1966; Barrett, 1977a; Price and Barrett, 1982; Barrett, Price and Shore 1983). In each tristylous species of *Eichhornia*, monomorphic populations occur and are associated with strong self-compatibility, weak pollen trimorphism, and modifications in reproductive structures which favour autogamy (Barrett, 1978, 1979, and unpublished data). While in *Eichhornia* tristyly is breaking down in some populations towards increased self-fertilization, the absence of floral modifications in *P. rotundifolia* and *P. cordata* suggest that the level of outcrossing is sufficient to maintain the tristylous genetic polymorphism.

Pontederia sagittata Presl. is a perennial, emergent aquatic which occurs commonly along the coastal plain of Mexico, Guatemala and Honduras (Lowden, 1973). The taxon has also been reported from scattered localities throughout Central and South America (Woodson and Schery, 1944; Castellanos, 1958). Populations occur in marshlands and estuaries, along drainage ditches and in low-lying pastures. Early taxonomic treatments of the genus included *P. sagittata* as a form (Solms-Laubach, 1883), or a variety (Woodson and Schery, 1944) of the widespread *P. cordata*. However, recent works have recognized the distinctive nature of *P. sagittata* with elevation to specific rank (Castellanos, 1958; Lowden, 1973). To our knowledge there are no published reports on the natural history of *P. sagittata* or of experimental work on the species.

This paper reports on field observations and experimental studies of Mexican populations of *P. sagittata*. Specifically we (1) describe the major morphological features of the tristylous syndrome of *P. sagittata*; (2) examine the expression of trimorphic incompatibility by controlled pollinations and observations of pollen tube growth and seed set; (3) document the population structure and patterns of pollen flow in natural populations. The data for *P. sagittata* is then compared with that available for *P. cordata* and *P. rotundifolia* to enable an evaluation of the general features of tristyly in the genus.

MATERIALS AND METHODS

Field observations and material for laboratory analysis were collected during July 1982, along the lowland coastal plain of Mexico in the states of Vera Cruz and Tabasco. *Pontederia sagittata* populations occur along the 500 km of highway from Jalapa to Villehermosa. Eight populations in this region were chosen for study. At Paso San Juan, Vera Cruz 1 and Vera Cruz 2 populations were in flooded pasture; at Minatitlan and Villehermosa they occurred in drainage ditches; at Laguna and Coatzacolcos in marshland; at Buena Vista the population bordered a river. Populations ranged in composition from dense monospecific stands to those in which *P. sagittata* was interspersed with grasses and other monocotyledonous aquatic plants. When field work was completed, rhizomes were transplated from the Vera Cruz 1 locality and cultivated in the University of Toronto glasshouses for experimental studies.

Flower and pollen measurements. To document the reciprocal positioning of anthers and stigmas, the height of these organs above the base of the ovary was measured on ten flowers per floral morph in five populations. Flowers were sampled from randomly chosen inflorescences. The size of anthers was determined under a dissecting microscope using preserved (standard FAA) flowers. The length and breadth of 60 anthers per morph was measured and compared by anther level using

ANOVA. The equatorial and polar axes of 100 pollen grains from each anther level per floral morph were measured with the aid of an ocular micrometer at × 40 power. This was undertaken on a collection of dry pollen, sampled from approximately five flowers from each of 20 inflorescences per floral morph from the Paso San Juan population, and for fresh pollen from glasshouse grown plants. Pollen production was estimated in five populations by collecting a single bud, which would have opened the following day, from five inflorescences per floral morph and preserving these in FAA. Anthers from each stamen level (15 total) were crushed in a known volume of lactophenol–glycerin containing cotton blue and the number of pollen grains was scored in eight haemacytometer counts for each sample. Pollen production was compared by anther level using *t*-tests.

Controlled pollinations. A crossing programme was undertaken in the glasshouse to determine the incompatibility relationships among floral morphs. Five clones of each morph were collected in the field from widely spaced plants to ensure that they represented different genetic individuals. The clones were then grown in plastic tubs at a temperature ranging from 20 to 35 °C with 12 h of light alternating with 12 h of darkness. Flowering commenced in September 1982, each clone producing from four to ten inflorescences between September and December 1982. Pollinations were performed each morning with fine forceps. It was not necessary to emasculate flowers prior to pollination as the seed set of undisturbed flowers was low (long-styled morph 0 % seed set, $n = 381$ flowers; mid-styled morph 3·2 %, $n = 324$; short-styled morph 2·4 %, $n = 253$). All flowers on a given inflorescence received the same pollination treatment. Treatments were: (1) legitimate cross-pollination, where pollen is transferred from anthers of one floral morph to the stigma of the same level in another floral morph; (2) intra-form illegitimate cross-pollination, where pollen from one anther level is placed on a stigma of a different clone of the same floral morph; (3) illegitimate self-pollination, where pollen is transferred from anthers to the stigma within a flower; (4) inter-form illegitimate cross-pollination where pollen from anthers of one floral morph is placed on a stigma at a different level in another floral morph. Approximately 1 month later, the mature fruit were collected and counted. As there is only one functional ovule in *P. sagittata*, fruit set is equivalent to seed set.

In an effort to examine the expression of self-incompatibility in *P. sagittata*, the behaviour of pollen tubes was observed. One, 4, 6, 8 and 24 h after self- or cross-pollinations were performed, the gynoecium of ten flowers per morph was removed and preserved in FAA. The temperature in the glasshouse during pollinations ranged from 25 to 30 °C. Styles were cleared in a solution of sodium hydroxide, stained in a 0·1 % solution of aniline blue (Martin, 1959), and observed with an epifluorescent microscope. Growth of pollen tubes was recorded relative to the style length after each time interval.

Field studies. To estimate the frequency of floral morphs in natural populations of *P. sagittata* eight populations were sampled. Between 75 and 100 inflorescences were selected at random along transects which traversed each population. Available evidence indicates that the floral morphs do not differ in inflorescence production and thus a sample of inflorescences is sufficient to estimate floral morph frequencies. Frequencies were compared by a G test for goodness-of-fit (Sokal and Rolf, 1969) to an isoplethic (1 : 1 : 1) equilibrium.

The patterns of pollen flow in four populations were investigated by estimating

the number of legitimate and illegitimate pollen grains deposited on naturally-pollinated stigmas. Flowers of *P. sagittata* last for 1 day, opening at approximately 0700 h at which time the anthers dehisce. Later anthers and filaments wither, and flowers close by 1430 h. Just prior to this time, 50 stigmas from 25 randomly selected inflorescences per floral morph were removed and preserved separately in FAA. Bulk collections of stigmas from each morph were later acetolysed (for method see Ganders, 1975; Price and Barrett, 1983) and samples of pollen grains suspended in a known volume of lactophenol–glycerin with cotton blue. Pollen was then measured and counted using a haemacytometer. In this manner, the relative proportions of the pollen morphs on each stigma type could be determined and compared to the relative contributions of the pollen morphs to the pollen pool of each population.

At each population an effort was made to collect all insect visitors to *P. sagittata* for later identification.

RESULTS

Unlike most heterostylous species, flowers of *P. sagittata* are zygomorphic. The showy lilac-coloured flowers are composed of six tepals which are fused for half their length into a perianth tube. A yellow nectar guide is present on the upper middle lobe. The ovary is superior, containing one functional and two abortive ovules. The androecium consists of six stamens adnate to the perianth tube. Flowers are born in a contracted, sheathed spike which arises from the rhizome on a stalk bearing one sagittate leaf. Inflorescences produce 70 to 220 flowers, each flower opening for a single day. An individual inflorescence bears flowers for an average of six consecutive days, and several to many inflorescences may be blooming at once in a single clone.

Morphological features of tristyly. In all populations examined, styles and stamens are well separated into three levels (Table 1). The floral morphs are distinct, with reproductive organs reciprocally arranged so the height of the stigma of a morph corresponds with one anther level of the other two morphs. The distance separating mid- from long-level organs is slightly greater than that separating short- from mid-level organs.

Differences were observed between the gynoecia of the floral morphs. The portion of the style just below the stigma is purple-pink in long- and mid-styled flowers and white in the short-styled morph. The long- and mid-styled morphs have stigmas which are lobed, the long-styled morph more deeply so, while the short-styled morph has a capitate stigma. The stigmatic papillae vary in length proportionately to style length and are spreading and least dense in the long-styled morph, and shortest and tightly clustered in the short-styled morph. Scanning electron micrographs of the papillae surface revealed no apparent differences among the morphs.

Anthers of *P. sagittata* fall into three size classes (Table 2). Long-level anthers are largest and distinct from mid-level anthers, which are intermediate in size. Short-level anthers are smallest. In our sample, the short-level anthers from the mid-styled morph were significantly smaller than those of the long-styled morph. Whether this difference is a consistent feature of the species requires further sampling.

In each of the floral morphs, the anther levels produce pollen grains of a

Table 1. *Mean height and standard deviation (mm) of stigmas and anthers in the three floral morphs of* Pontederia sagittata

Population	Organ level*	Floral morph		
		Long	Mid	Short
Paso San Juan	l	14·9±0·7	14·4±0·4	14·7±0·7
	m	8·8±0·6	9·0±0·6	9·0±0·6
	s	4·7±0·6	4·7±0·6	3·7±0·4
Vera Cruz 1	l	14·3±07	14·1±0·9	14·1±1·0
	m	9·1±0·4	8·8±0·8	8·1±0·9
	s	4·5±0·7	4·7±0·4	3·9±0·2
Coatzacolcos	l	12·6±0·9	12·8±0·9	12·8±0·6
	m	8·1±0·4	7·8±0·9	8·1±0·5
	s	4·0±0·5	4·2±0·6	3·4±0·4
Villehermosa	l	13·6±0·9	13·7±0·5	13·5±0·5
	m	8·4±0·4	8·1±0·5	8·7±0·6
	s	4·5±0·8	4·9±0·5	3·8±0·3
Laguna	l	15·3±0·7	15·1±0·9	14·4±0·6
	m	8·5±0·6	9·1±0·8	9·1±0·7
	s	5·0±0·8	3·0±0·5	3·7±0·4

*l, Long; m, mid; s, short.
Each value is based on ten flowers per morph.
Underlined figures are stigma heights and the remainder anther heights.

Table 2. *Mean length × width with standard deviation (mm) of anthers in floral morphs of* Pontederia sagittata *grown under glasshouse conditions*

Anther level*	Floral morph		
	Long	Mid	Short
l	—	1·09ᵃ±0·16 × 0·63±0·06	1·06ᵃ±0·07 × 0·61±0·05
m	0·87ᵇ±0·09 × 0·56±0·06	—	0·86ᵇ±0·07 × 0·61±0·04
s	0·77ᶜ±0·04 × 0·53±0·07	0·69ᵈ±0·06 × 0·47±0·07	—

Means not significantly different if superscripts (a, b, c, d) are the same. Means compared by SNK *a posteriori* procedure at the 0·05 level following ANOVA. ($F = 136·4$; D.F. $= 2, 84$, $P < 0·001$).
*l, Long; m, mid; s, short.
Each value is based on three anthers from 15 flowers per morph ($n = 45$).

distinctive size (Table 3). Long-level anthers contain the largest pollen grains, mid-level anthers contain pollen grains which are intermediate in size and the smallest pollen grains are produced by short-level anthers. There is no significant difference between the size of the pollen grains from equivalent levels of different floral morphs. Thus in *P. sagittata* pollen trimorphism is complete, with no overlap in the size of pollen grains from different anther levels (Fig. 1).

Pollen production tends to be inversely correlated with anther level (Table 4). Long-level anthers produce the largest pollen grains and contain approximately one-quarter the number of pollen grains found at short-level anthers. There is no significant difference between the number of pollen grains produced by long-level anthers of mid- and short-styled flowers. However, mid-level anthers of the short-styled morph on average produce 2·1 times more pollen grains than mid-level anthers of long-styled flowers. This difference in pollen production at mid-level

Table 3. *Mean equatorial × polar axis with standard deviation (μm) of pollen grains of the floral morphs of* Pontederia sagittata *from Vera Cruz, Mexico.*

Anther level*	Floral morph		
	Long	Mid	Short
l	—	66·8 ± 4·2 × 25·7 ± 3·7	69·9 ± 3·7 × 26·5 ± 3·7
m	54·0 ± 2·9 × 19·7 ± 1·7	—	53·9 ± 2·8 × 20·8 ± 2·4
s	32·4 ± 2·4 × 12·9 ± 1·8	31·5 ± 2·1 × 13·0 ± 1·6	—

*l, Long; m, mid; s, short.
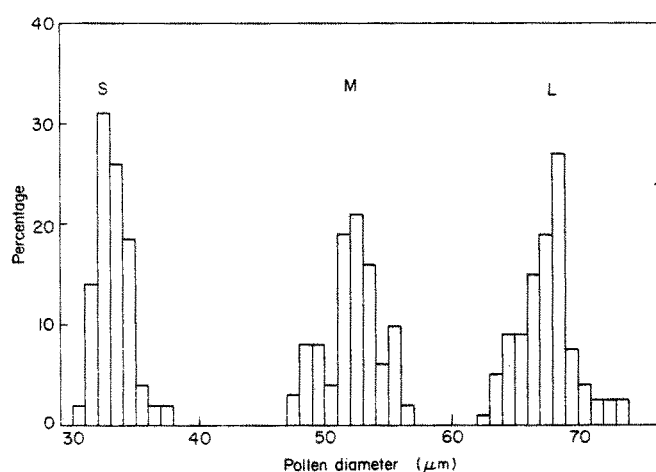Each value is based on 100 grains from 20 flowers per morph.

Fig. 1. Pollen trimorphism in *Pontederia sagittata* from Vera Cruz, Mexico. (*n* = 100 pollen grains per anther level). L, long; M, mid; S, short floral morphs.

anthers is significant (*t* = 2·7, d.f. = 4; *P* < 0·05), despite variation between populations in total pollen production among the morphs (Table 4). Examination of the pollen production data in Table 4 illustrates that both long- and mid-styled flowers produce more than twice the quantity of pollen grains produced by short-styled flowers.

The expression if incompatibility. The results of controlled pollinations on 15 clones of *P. sagittata* are presented in Table 5. The presence of a self-incompatibility system in all clones of *P. sagittata* is demonstrated by reduced seed set following self-pollination, in comparison with legitimate cross-pollination (Fig. 2). The behaviour of pollen from the two anther levels was markedly different in self-pollinations. For both the long- and short-styled morphs, pollination with the nearest (mid) anther level was moderately productive of seed. In comparison, pollen from short-level anthers of the long-styled morph and long-level anthers of the short-styled morph produced almost no seed. The mid-styled morph exhibited weak self-incompatibility when pollen from long-level anthers was applied to mid-level stigmas, whereas pollen from short-level anthers resulted in low seed set.

Differences between the floral morphs in the strength of self-incompatibility are

Table 4. *Pollen production (with coefficient of variation) in five populations of Pontederia sagittata from Mexico*

| | Anther level of floral morph | | | | | |
| | Long-level anthers | | Mid-level anthers | | Short-level anthers | |
Population	M	S	L	S	L	M
Vera Cruz 1	2222 (0·12)*	1333 (0·24)	1214 (0·31)	3081 (0·16)	7443 (0·15)	11258 (0·11)
Paso San Juan	2681 (0·28)	2073 (0·29)	2044 (0·17)	3555 (0·16)	9524 (0·06)	8828 (0·10)
Buena Vista	1851 (0·11)	2325 (0·19)	1733 (0·17)	3747 (0·16)	9791 (0·11)	11213 (0·11)
Minatitlan	3674 (0·17)	3170 (0·30)	1549 (0·12)	4622 (0·16)	13096 (0·06)	15568 (0·10)
Laguna	3170 (0·13)	2029 (0·15)	2751 (0·15)	4457 (0·16)	11466 (0·19)	12711 (0·10)
Mean	2719	2186	1858	3892	10264	11915
s.d.	727	661	582	641	2132	2471
t	0·901		2·74		0·852	
P	n.s.		<0·05		n.s.	

*Long-level anthers of mid-styled morph.
Each value is based on 15 anthers from five flowers.

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Table 5. *Seed set after controlled pollinations of* Pontederia sagittata *under glasshouse conditions*

Pollination treatment*	No. clones	No. inflorescences	No. flowers pollinated	No. flowers producing fruit	Per cent flowers producing fruit
Illegitimate pollinations					
L × s/L (self)	4	4	613	3	0·5
L × s/M (cross)	3	3	193	2	1·0
L × m/L (self)	5	8	1066	129	12·1
L × m/L (cross)	4	4	481	59	12·3
L × m/S (cross)	4	4	437	34	7·8
M × s/M (self)	4	4	505	90	17·8
M × s/L (cross)	4	4	241	36	14·9
M × l/M (self)	5	12	1834	1420	77·4
M × l/M (cross)	5	5	798	596	74·6
M × l/S (cross)	5	7	512	323	63·1
S × l/S (self)	5	5	457	43	9·4
S × l/M (cross)	2	2	126	3	2·3
S × m/S (self)	5	6	862	217	25·1
S × m/S (cross)	4	4	475	127	26·7
S × m/L (cross)	3	3	179	60	33·5
Legitimate pollinations					
L × l/M or S	5	6	795	646	81·2
M × m/L or S	5	6	869	789	91·8
S × s/M or L	5	5	654	513	78·4

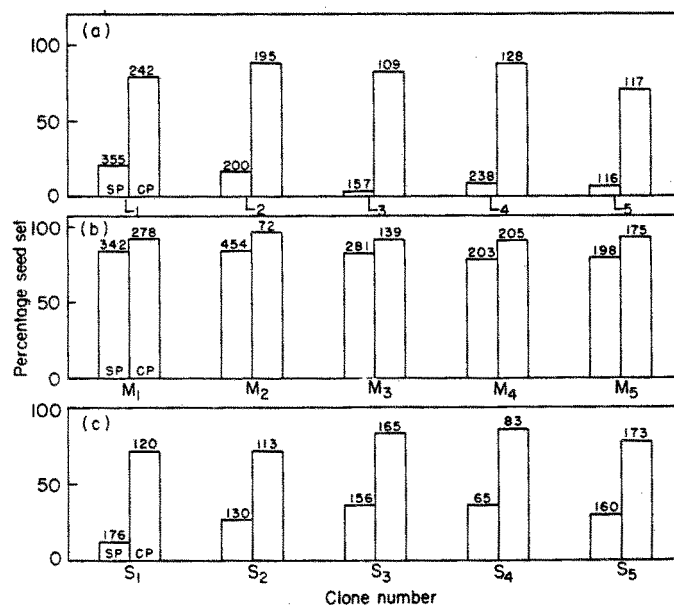* Style length (♀) × anther level/style length (♂).

Fig. 2. Percentage seed set following self-pollination (SP) and cross-pollination (CP) of 15 clones of *Pontederia sagittata* under glasshouse conditions. (a) Long-styled, (b) mid-styled, (c) short-styled morph. The number of flowers pollinated in each clone is indicated. The clones were obtained from a population at Vera Cruz, Mexico.

evident from the crossing programme. In general, the long- and short-styled morphs exhibited strong self-incompatibility. Although the short-styled morph appears less self-incompatible than the long-styled morph, a more extensive crossing programme involving more clones would be needed to confirm this. Differences in the expression of self-incompatibility between clones suggests that there may exist genetic variation for this trait, but again, further studies are required to determine the basis of this variation.

Results from within-form illegitimate crosses reveal the complexity of the tristylous breeding system. The floral morphs differ in the expression of cross-incompatibility in a similar manner as in self-incompatibility (Table 5). Mid-styled clones are the least incompatible, while strong incompatibility is exhibited by the short- and long-styled morphs. In addition, for all three floral morphs, seed set for within-form illegitimate crosses is very similar to that obtained from self-pollination with the same anther level. Inter-form illegitimate pollinations confirm the difference between the floral morphs in the expression of cross-incompatibility. Seed set from both inter-form combinations was in accord with self- and intra-form illegitimate pollinations (Table 5).

The observed variation in seed set following controlled pollinations is associated with differences in the growth rate of pollen tubes in the style. Because of the large number of pollen grains clumped on stigmas following pollination, germination could not be reliably quantified. However, in both self- and legitimate-pollination treatments, large numbers of germinating pollen grains were observed on all stigmas. The fluorescence of callose plugs, laid down along the length of pollen tubes, enables their growth in the style to be followed.

Pollen tubes resulting from legitimate pollinations began growth towards the stylar base soon after application. Within 1 h, pollen tubes had descended about one-half the stylar length in long- and short-styled flowers, and in some cases had reached the base in mid-styled flowers. After 4 h many pollen tubes had reached the base of the style in all three floral morphs.

Pollen tubes of self pollen from the most compatible anther level (long-level in the mid-styled morph and mid-level in the short- and long-styled morphs) grew more slowly in the short-and long-styled morphs in comparison with pollen tubes from legitimate pollinations. No difference between long (illegitimate) and mid (legitimate) pollen tube behaviour was detected in the mid-styled morph for any of the time intervals. After 1 h, self pollen tubes had not penetrated the stigmas of short-styled flowers. However, after 4, 6 and 8 h, many pollen tubes had grown to the base of the style. In the long-styled morph, mid pollen tubes had grown one-half to three-quarters the stylar distance 4 h after pollination. Within 6 h, in half of the styles examined, one or two pollen tubes had reached the base, while they were at most three-quarters of this distance in the remaining five styles which were examined. Many illegitimate pollen tubes were observed at the base of long styles after 8 h.

Self-pollination with strongly incompatible pollen from alternate anther levels resulted in more pronounced inhibition of pollen tube growth. The combination of short styles with pollen from long-level anthers behaved variably. Three hours after pollination, pollen tubes had reached the base of the style in some flowers whereas in others they had not penetrated the stigmatic tissue. Pollen tubes in long- and mid-styled morphs terminated growth before reaching the base of the style. Twenty-four hours after pollen from short-level anthers was applied to stigmas of long-styled flowers, most pollen tubes had germinated and penetrated the

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stigma. However, in the ten styles examined, all were less than one-half of the total stylar length. Similarly, pollen from short-level anthers germinated profusely on stigmas of mid-styled flowers, but after 24 h in only one case had a pollen tube reached the base of the style, all others were inhibited at aproximately one-third of this distance.

Table 6. *Floral morph frequencies in natural populations of* Pontederia sagittata *from Mexico*

| Population | Floral morph | | | n | G^* | P |
	Long	Mid	Short			
Paso San Juan	0·361	0·248	0·391	105	3·97	n.s.
Vera Cruz 1	0·193	0·451	0·354	124	13·67	< 0·01
Vera Cruz 2	0·310	0·373	0·316	142	1·29	n.s.
Coatzacolcos	0·120	0·378	0·501	114	25·51	< 0·001
Minatitlan	0·355	0·355	0·290	76	0·82	n.s.
Villehermosa	0·392	0·299	0·308	107	1·87	n.s.
Laguna	0·270	0·380	0·350	96	1·99	n.s.
Buena Vista	0·270	0·354	0·375	102	1·89	n.s.
Total	—	—	—	—	48·97	< 0·01
Pooled	0·281	0·359	0·359	866	12·40	< 0·01
Heterogeneity	—	—	—	—	36·57	< 0·01

* Depature from 1:1:1.

Population structure. The three floral morphs were represented in each of the eight populations sampled (Table 6). Six populations did not differ significantly from an isoplethic equilibrium (equal proportions of the three morphs). Two populations, Vera Cruz 1 and Coatzacolcos, were anisoplethic (unequal proportions of the three morphs). A test for heterogeneity indicates that populations differ significantly from one another in floral morph frequencies, and there is a tendency for the long-styled morph to be under-represented. When all localities are pooled, the G statistic reveals departure from an isoplethic equilibrium.

Pollen flow. Pollen of *P. sagittata* exhibits a striking trimorphism (Fig. 1), which enables the source (anther level) from which pollen grains originated to be identified unambiguously. Unequal representation of the style morphs and differences between the morphs in the number of flowers per inflorescence will affect the contribution of each pollen type to the pollen pool of the population. The average number of open flowers per inflorescence per day did not differ between style morphs in three populations where this parameter was measured: Paso San Juan, L = 36·8, M = 34·9, S = 29·1 (F = 1·31, d.f. = 2, 60, n.s.); Vera Cruz, L = 24·6, M = 23·1, S = 27·1 (F = 1·18, d.f. = 2, 44, n.s.); and Buena Vista, L = 4·2, M = 35·1, S = 41·6 (F = 0·80, d.f. = 2, 44, n.s.) where L = long-, M = mid- and S = short-styled morphs. Table 7 gives the relative contributions of the pollen morphs, following adjustment for floral morph frequency and pollen production, in populations where pollen flow was examined. Variation among the populations is due primarily to differences in pollen production of the morphs. In all cases pollen from short-level anthers comprises more than one-half of the available male gametes, and pollen from mid-level anthers contributes on average slightly more than pollen from long-level anthers.

Table 7. *Per cent contribution of the three pollen morphs to the pollen pool of natural populations of* Pontederia sagittata *from Mexico*

| | Pollen type* | | | |
Population	l	m	s	Total
Buena Vista	15·1	18·7	66·2	100
Laguna	15·8	19·2	65·0	100
Paso San Juan	16·0	23·0	61·0	100
Minatitlan	15·6	13·2	71·2	100
Average	15·6	18·5	65·9	

* l, Long pollen; m, mid pollen; s, short pollen.

In each population, stigmas were acetolysed by morph in batches of 50. Thus, variation among stigmatic pollen loads of individual flowers cannot be detected. The average total pollen load per stigma ranged from 50·5 (Paso San Juan) to 149·1 (Minatitlan) grains. Floral morphs appear to differ in the number of pollen grains deposited on stigmas. Flowers of the mid-styled morph received more pollen grains than the long- and short-styled morphs in three of the four populations, while stigmas of the short-styled morph received the lowest number of pollen grains in three of the four populations.

If pollination in populatons of *P. sagittata* was random, the pollen morphs would be expected to be deposited on stigmas in the same proportion as they are produced in the population. Accordingly, random pollen loads were calculated for each locality. Observed loads (an average value per stigma) are compared to this random expectation in Table 8. Deviations from random pollination are of interest. In all populations, the long-styled morph received more legitimate pollen grains than would be predicted from random pollination (Table 8). At Paso San Juan and Laguna, the mid-and short-styled morphs exhibited pollen loads which did not differ from random expectations. At Minatitlan, the mid-styled morph received a greater number of legitimate pollen grains than would be expected from random pollination while the short-styled morph received excess amounts of illegitimate pollen. At Buena Vista the reverse pattern was evident in these morphs.

At all localities sampled the long-styled morph received more legitimate pollen grains than would be expected from random pollination. Nevertheless, the mean per cent of the total stigmatic load which was composed of pollen from long-level anthers was less than 50 % (Table 9). This is a result of the relatively low number of pollen grains contributed by long-level anthers. In contrast, the pollen load of short-styled flowers was composed of greater than 50 % legitimate pollen in all four populations, however in only one locality (Buena Vista), was this in excess of random expectation. Thus, the great differences in the contribution of the pollen morphs to the pollen pool of *P. sagittata* populations have a significant influence on variation among the floral morphs in the number of compatible grains deposited on stigmas.

In an effort to overcome difficulties of interpretation of pollen flow data, Ganders (1974) devised an 'index of efficiency' for evaluating the effectiveness of heterostyly in promoting legitimate pollination. The index is $E = o - r/d - r$, where o is the observed frequency of one of the pollen morphs, r is the expected frequency of the pollen morph with random pollination, and d is the expected frequency of the

Table 8. *Mean pollen deposition* (n = 50 *stigmas for each morph*) *on stigmas of* Pontederia sagittata *from natural populations in Mexico*

Population	Style morph		Pollen type[†]			G[‡]
			l	m	s	
Buena Vista	Long	Observed	20·8	31·9	47·2	14·6*
		Expected	15·1	18·7	66·2	
	Mid	Observed	5·55	7·77	124·4	42·4*
		Expected	20·9	25·7	91·2	
	Short	Observed	3·33	5·55	88·9	32·6*
		Expected	14·8	18·2	64·8	
Laguna	Long	Observed	17·4	3·11	4·75	36·9*
		Expected	3·98	4·85	16·4	
	Mid	Observed	11·1	30·8	92·6	5·52
		Expected	21·2	25·8	87·4	
	Short	Observed	3·33	13·3	28·9	4·40
		Expected	7·18	8·74	29·6	
Paso San Juan	Long	Observed	27·7	13·3	18·8	32·6*
		Expected	9·6	13·8	36·5	
	Mid	Observed	4·4	7·3	41·1	5·38
		Expected	8·5	12·3	32·5	
	Short	Observed	8·8	10·0	20·0	3·28
		Expected	6·2	9·0	23·7	
Minatitlan	Long	Observed	55·6	43·3	92·2	42·4*
		Expected	29·7	25·3	136·0	
	Mid	Observed	17·7	46·7	146·6	16·2*
		Expected	32·8	27·9	150·2	
	Short	Observed	3·33	14·4	27·7	11·3*
		Expected	7·09	6·02	32·4	

* $P < 0.05$
[†] l, Long-level anther; m, mid-level anther; s, short-level anther.
[‡] Departure from random expectation.
Expected values are assuming random pollination.

Table 9. *Levels of legitimate* (a) *and illegitimate* (b) *pollen capture by stigmas of* Pontederia sagittata *from natural populations in Mexico*

| Population | Average no. of pollen grains per stigma* | Floral morph | | | | | |
| | | Long | | Mid | | Short | |
		(a)	(b)	(a)	(b)	(a)	(b)
Buena Vista	111·8	20·8	79·2	5·6	94·4	90·9	9·1
Laguna	68·5	68·9	31·1	22·9	77·1	63·4	36·6
Paso San Juan	50·5	46·3	53·7	14·6	85·4	51·4	48·6
Minatitlan	149·1	29·1	70·9	22·1	77·9	61·0	39·0
Average		41·3	58·7	16·3	83·7	60·7	33·3

* Based on eight haemacytometer counts.
Values are expressed as percentages of the total pollen load.

pollen morph with legitimate pollination. Thus zero efficiency is complete random pollination, and 100% efficiency is complete legitimate pollination. Negative values may be obtained, e.g. -100% efficiency is total illegitimate pollination. This approach was used for the pollen flow data collected from tristylous populations of *P. sagittata*. Table 10 gives measures of the 'efficiency' of legitimate

Table 10. '*Efficiency' of legitimate pollination in natural populations of* Pontederia sagittata *from Mexico*

Population	Floral morph		
	Long	Mid	Short
Buena Vista	6·6	−15·9	73·1
Laguna	63·0	4·6	−4·7
Paso San Juan	35·9	−11·0	−24·4
Minatitlan	16·0	10·2	−35·6

Values expressed as percentages; negative values indicate illegitimate pollination.

pollination in each floral morph. Except for the short-styled morph at Buena Vista, the long-styled morph is considerably more effective than the other floral morphs in capturing legitimate pollen. All morphs, but particularly the short-styled morph, exhibited considerable inter-population variation in patterns of pollen deposition.

Insect visitors. The behaviour and morphological features of pollinators influence their ability to mediate legitimate pollination. Observation and collection of insects revealed that in seven of eight populations only a single visitor was consistently present. This was the syrphid fly, *Lycastrirlynca willistoni* Coquillett. It was abundant from early morning until flower closure in all populations visited. *Lycastrirlynca willistoni* possesses a very long slender probiscis, allowing it to collect nectar from the base of the corolla tube of *P. sagittata*. In a single population (Laguna) the long-tongued solitary bee, *Florilegus condignus* Cresson, of the family Anthophoridae, was a common visitor to flowers.

DISCUSSION

The general morphological and physiological characteristics of tristyly in *P. sagittata* are similar to those of trimorphic taxa in the Lythraceae and Oxalidaceae. Self-incompatibility and size trimorphism of pollen are associated with a reciprocal arrangement of reproductive organs in the floral morphs. Pollen diameter and anther size increase with stamen height, while the amount of pollen produced by the three anther levels is negatively correlated with stamen height. Several of the unusual features of the tristylous syndrome of *P. cordata* (Price and Barrett, 1982) are present in *P. sagittata*. Although the species differ in total pollen production per flower, both possess variations in production between the mid-level anthers of the long- and short-styled morphs. Mid-level anthers of the short-styled morph produce approximately twice the number of pollen grains found in mid-level anthers of the long-styled morph. In *P. cordata* this difference is associated with an anther size dimorphism; mid-level anthers of the short-styled morph are significantly larger than those of the long-styled morph (Price and Barrett, 1982). However in *P. sagittata*, mid-level anthers are uniform in size indicating that anther size is not necessarily a determinant of pollen grain number. Within both species, pollen produced by mid-level anthers of the two floral morphs is similar in size. An examination of stamen development is in progress in an effort to reveal the developmental basis of these complex patterns of pollen production. The

implications of these differences to the mating systems of populations are treated in Barrett *et al.* (1983).

Pontederia sagittata is the third species of the genus for which the incompatibility relationships of the floral morphs have been studied experimentally. Data obtained in this study are similar to those for *P. cordata* (Ornduff, 1966; Barrett and Anderson, unpublished data) and *P. rotundifolia* (Barrett, 1977a). A physiological self-incompatibility system restricts the amount of seed produced from self-pollinations. The most striking feature of data for the *Pontederia* species concerns the weak expression of incompatibility in the mid-styled morph. Self-, intraform- and interform-pollinations with pollen from long-level anthers are highly compatible. Values for seed set following self-pollination of the three species are: *P. cordata* 53·6 % (Ornduff, 1966) and 58·6 % (Barrett and Anderson, unpublished data), *P. rotundifolia* 68·6 % (Barrett, 1977a) and *P. sagittata* 77·4 %. In the long- and short-styled morphs of these species self-incompatibility is more strongly developed. Elsewhere among genera with trimorphic incompatibility, weak self-incompatibility in the mid-styled morph has been reported in *Lythrum salicaria* (Darwin, 1877; Stout, 1923), *Oxalis* spp. (Ornduff, 1972) and *Biophytum sensitivum* (Mayura Devi, 1964).

The genetic and physiological basis of differences among the floral morphs in the expression of self-incompatibility is obscure. Modifier genes linked to the dominant M allele at the locus governing mid-style length may be involved. Alternatively weak self-incompatibility may be a pleiotropic effect of genes which directly control the expression of tristylic characters such as pollen size or style length. To what extent the self-compatibility of the mid-styled morph influences its mating system is uknown. Computer simulation models of increased selfing of the mid-styled morph in initially tristylous populations indicate that it may spread to fixation (Charlesworth, 1979; Barrett *et al.*, 1983). Interestingly in self-compatible, tristylous species of *Eichhornia*, populations containing a single morph are most commonly composed of the mid-styled morph (Barrett, 1977b; Barrett and Forno, 1982) and the breakdown of tristyly via homostyle formation most frequently involves floral modification of this morph (Barrett, 1979 and unpublished data). Self-compatibility, as well as a greater propensity for self-pollination in comparison with the long- and short-styled morphs (see Charlesworth, 1979), may favour this form at low population densities during colonizing episodes.

While the occurrence of self-compatibility in the mid-styled morph of *Pontederia* spp. may provide clues to the initial conditions involved in the evolutionary breakdown of tristyly, it should be noted that no floral modifications were observed in field populations of *P. sagittata*. In fact, their size, and the relative frequency of floral morphs in populations, suggests that the mating system is primarily outcrossed. If this is true, offspring resulting from self-fertilization would be expected to show inbreeding depression. We are currently making comparisons of the growth and reproductive performance of offspring, resulting from controlled self- and cross-pollinations, to test this hypothesis.

Heterostylous species exhibit sporophytic control of self-incompatibility. Unlike homomorphic sporophytic systems where trinucleate pollen and stigmatic inhibition of incompatible pollen are common, heteromorphic species may possess binucleate (*Forsythia, Primula, Pontederia*) or trinucleate (*Amsinckia, Limonium, Linum*) pollen (see Brewbaker, 1957). Inhibition may be due to failure of pollen to germinate on the stigma as in *Primula veris* (Richards and Ibrahim, 1982), germination but abnormal pollen tube growth with little or no penetration of

stigmatic tissue e.g. *Primula vulgaris* (Shivanna, Heslop-Harrison and Heslop-Harrison, 1981), slow and incomplete growth of pollen tubes in the style e.g. *Primula obconica* (Stevens and Murray, 1982) or some form of ovarian inhibition (Schou and Philipp, 1983; and see below). In addition the site of inhibition may differ between the floral morphs (Lewis, 1943; Richards and Ibrahim, 1982; Bawa and Beach, 1983). Thus it appears that the mechanisms which reduce illegitimate fertilizations may vary considerably among heteromorphic taxa.

There have been few studies of the inhibition site in tristylous species. In *Lythrum salicaria*, Esser (1953) demonstrated that illegitimate pollen tubes penetrated the stigma and terminated growth in the style. The floral morphs differed in the distance, relative to the style length, attained by illegitimate pollen tubes. In addition, the total growth of pollen tubes from pollinations involving the two illegitimate anther levels differed in the long- and mid-styled morphs. In illegitimate combinations there were no differences between the behaviour of pollen tubes from equivalent anther levels of separate style morphs. In *L. junceum*, incompatible pollen tubes of the three floral morphs also exhibit stylar inhibition, and in general do not grow beyond half the length of the style (Dulberger, 1970a).

. Incompatible pollen germinated readily and penetrated the stigma in self-pollinations of *P. sagittata*. In most comparisons. illegitimate pollen tubes grew more slowly than legitimate pollen tubes. In strongly incompatible pollinations, pollen tubes had not reached the base of the style after 24 h. However, in several classes of illegitimate pollination (e.g. Lxm/L, Sxm/S), pollen tubes were regularly observed at the base of the style after a relatively short period of time (8 h). Corresponding seed set data for these pollen–pistil combinations indicate that only a few seeds matured. This suggests that the incompatibility reaction may be occurring, at least in part, after pollen tubes have entered the ovary. Alternatively, a post-zygotic reaction, as has been reported in *Theobroma cacao* (Cope, 1962), *Borago officinalis* (Crowe, 1971), and the heteromorphic taxa *Narcissus tazetta* (Dulberger, 1964), and *Anchusa officinalis* (Schou and Philip, 1983), cannot be ruled out. Low seed set as a result of post-zygotic phenomena may be due to incompatibility mechanisms and/or inbreeding depression (Brink and Cooper, 1947; Lloyd, 1968). In *P. sagittata* illegitimate *cross*-pollination yielded levels of seed set similar to those from self-pollination. Therefore, inbreeding depression is unlikely to be involved. It remains to be determined if some form of ovarian incompatibility is operating in *P. sagittata* as well as pollen tube inhibition in the style.

Darwin (1877) proposed that the adaptive significance of tristyly is to promote insect-mediated pollination among floral morphs with anthers and stigmas at equivalent levels (legitimate pollination). The marked size trimorphism of pollen in *Pontederia* species enables an evaluation of this hypothesis (Price and Barrett, 1982, 1983). Although considerable variation in the amount and composition of pollen on naturally pollinated stigmas of *P. sagittata* was recorded, the trends which emerged are in accord with pollen flow data from *P. cordata* (Price and Barrett, 1983). In both species the likelihood of legitimate pollination appears to be highest in the long-styled morph and lowest in the short-styled morph. In addition the total amount of pollen deposited on stigmas tends to be greatest in the mid-styled morph. The pollen flow data for *Pontederia* are in striking contrast to those for distylous species (reviewed by Ganders, 1979) where, in general, the stigmas of the long-styled morph experience greater illegitimate pollination than those of short-styled flowers. The factors which could account for these asym-

metries among floral morphs in pollen flow patterns, as well as variation among populations, are many and include; the abundance and type of insect visitors, their foraging and grooming behaviour, the spatial pattern and density of floral morphs, and the adherence properties of the pollen morphs to the insect as well as to the stigmatic papillae of the floral morphs. A major challenge to pollination biologists interested in heterostylous plants will be to devise experimental techniques to distinguish the relative importance of these influences under field conditions.

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